REMARKS

Claims 1-49, as listed in the Amendment and Response filed May 23, 2006, are pending in this application. Claims 20-49 are withdrawn from consideration, as a result of the restriction requirement imposed in the Office Action mailed November 1, 2005.

The Rejections of Claims 1-19 under 35 U.S.C. § 102

Claims 1-19 are rejected as being anticipated by Diaz-Collier *et al.*, EPO publication EP 0 559 632 A1 ("Diaz-Collier"). Applicants respectfully traverse these rejections.

Independent claims 1 and 10 (and their dependent claims 2-9 and 11-18, respectively) recite purified preparations and pharmaceutical compositions comprising a plurality of TFPI or TFPI analog molecules. Less than about 12% of the TFPI or TFPI analog molecules are modified species. Modified species have one or more of the following modifications: oxidation, carbamylation, deamidation, cysteine adducts, aggregation, and misfolding. In addition to these properties, independent claim 19 recites a pharmaceutical formulation comprising 20 mM sodium citrate, 300 mM L-arginine, and 5 mM methionine at pH 5.5.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Diaz-Collier does not meet the legal standard for either express or inherent anticipation.

Diaz-Collier does not expressly disclose the amounts of oxidized, carbamylated, deamidated, cysteine adduct, aggregated, and/or misfolded species present. Moreover, it is black letter law that inherent anticipation requires that the inherent element necessarily is present in the single reference:

> a prior art reference may anticipate without disclosing a feature of the claimed invention if that characteristic is necessarily present, or inherent, in the single anticipating reference.

Schering Corp. v. Geneva Pharm., Inc., 339 F.3d 1373, 1377 (Fed. Cir. 2003) (emphasis added). Diaz-Collier does not necessarily (or inherently) disclose purified preparations or pharmaceutical compositions comprising TFPI or TFPI analog molecules "wherein less than about 12% of the TFPI or TFPI analog molecules are modified species [oxidized, carbamylated, deamidated, cysteine adduct, aggregated, and/or misfolded]" as recited in independent claims 1, 10, and 19. Diaz-Collier therefore is not an inherently anticipating reference.

The Invention

Applicants' invention is directed to highly purified preparations of TFPI and TFPI analogs. Specification, Abstract. This invention is based on the discovery of a defined sequence of chromatography and other operations, following the refolding of TFPI or TFPI analogs expressed in *E. coli*. These operations result in a final preparation containing less than about 12% of modified species, as claimed. Specification, paragraph [64]. In particular, after refolding, TFPI or TFPI analogs are purified by (A) SP-Sepharose fast flow (FF) chromatography, (B) a first concentration/diafiltration step, (C) Q-Sepharose high performance (HP) chromatography, (D) butyl hydrophobic interaction chromatography (HIC), (E) SP-Sepharose high performance (HP) chromatography, and (F) a second concentration/diafiltration step. Specification, paragraph [62].

In working examples, Applicants have illustrated the significantly reduced amounts of modified species obtained with this sequence of operations, relative to known processes. Specification, paragraph [65] and Table 1. The purification process that Applicants have discovered provides TFPI and TFPI analog preparations containing fewer modified species than

the methods described in Gustafson *et al.*, PROTEIN EXPRESSION AND PURIFICATION 5: 233-41 (1994), as well as in the following patent publications: WO 96/40784; U.S. Patent No. 6,319,896; and U.S. Patent No. 6,323,326. Specification, paragraph [65]. Applicants' claimed method was developed during extensive research to provide pharmaceutical compositions comprising TFPI or TFPI analogs which would meet applicable FDA standards for purity in Phase III clinical trials. These standards were not achieved with prior art methods.

EP 0 559 632 A1 ("Diaz-Collier")

The disclosure of Diaz-Collier, applied against the pending claims, is directed to an *E. coli* expression system for obtaining TFPI having a high level of specific activity compared to TFPI derived from mammalian cell (*e.g.*, human SK hepatoma cell) hosts. Page 2, line 52 to page 3, line 2 and page 3, lines 10-16. In particular, Diaz-Collier discloses purification measures before refolding, which result in improved TFPI activity. According to Diaz-Collier,

> Unexpectedly, purification of TFPI prior to the refolding step by sulfonation followed by anion exchange chromatography according to embodiment A of the method of the invention resulted in a two-fold higher specific activity than an highly purified full-length SK hepatoma-derived TFPI in the inhibition of tissue factor-induced coagulation time assay . . .

Page 2, lines 53-57. Diaz-Collier discloses the use of both anion exchange chromatography (embodiment A) and cation exchange chromatography (embodiment B) prior to refolding. See, for example, page 2, lines 39-48 and also page 9, lines 1-2: "Therefore, attempts were made to purify TFPI prior to [the] refolding step, by sulfonation followed by anion exchange chromatography. . .".

In addition to higher activity, Diaz-Collier also reports a TFPI product having a "greater level of homogeneity" relative to SK hepatoma-derived material. Page 3, lines 10-11. This is

due to the following, specifically identified types of heterogeneity which result from mammalian expression systems and not *E. coli* expression systems: A. Phosphorylation, B. Proteolytic Internal Cleavages, and C. Glycosylation. Page 3, lines 18-39.

The invention of Diaz-Collier therefore resides in the ability to produce active TFPI from *E. coli* expression systems, using the disclosed pre-refolding purification steps. Expression from *E. coli* advantageously reduces TFPI phosphorylation, internal proteolytic cleavages, and glycosylation, which Diaz-Collier refers to as "classes of heterogeneity." Page 3, line 15.

DIAZ-COLLIER'S DISCLOSED PROCESS DOES NOT
INHERENTLY RESULT IN THE CLAIMED TFPI COMPOSITION

As stated above, Diaz-Collier provides TFPI preparations that are derived from *E. coli* expression systems, are highly active, and have reduced "heterogeneity." Diaz-Collier specifically identifies "heterogeneous" TFPI species as those which are phosphorylated, internally cleaved, and glycosylated in mammalian cell expression systems. Diaz-Collier however is completely silent with respect to reducing amounts of oxidized, carbamylated, deamidated, cysteine adduct, aggregated, and/or misfolded TFPI molecules, let alone achieving less than about 12% of these modified species, as claimed.

The Office Action cites the following passage describing Diaz-Collier's electrospray analysis: "the material produced from the EXAMPLE 1 process appeared to be essentially homogeneous (>95%)." Page 12, lines 1-2. According to Diaz-Collier's definition of "heterogeneous" above, a >95% homogeneous TFPI preparation would contain fewer than 5% phosphorylated, internally cleaved, or glycosylated TFPI species. However, Diaz-Collier does not mention the extent of oxidized, carbamylated, deamidated, cysteine adduct, aggregated, and/or misfolded TFPI molecules. Nor does Diaz-Collier suggest improving TFPI preparation steps after refolding to reduce these modified species.

In fact, Diaz-Collier discloses only one post-refolding purification step, namely cation exchange chromatography. See step (4) of embodiments A and B, page 2, lines 42 and 48. More recent TFPI preparation methods have added purification steps, after refolding, to improve the final product purity. For example, Gustafson *et al.* describe "three post-refold cation exchange steps." Page 239, right-hand column, of PROTEIN EXPRESSION AND PURIFICATION 5: 233-41 (1994) ("Gustafson"). Even so, the purification method that Applicants have now discovered "produces preparations of TFPI or TFPI analog molecules that contain fewer modified TFPI or TFPI analog species than previous purification methods described in [Gustafson]." Specification, paragraph [65].

Moreover, as explained in detail in the Amendment and Response filed May 23, 2006, the electrospray analysis conducted in Diaz-Collier determines molecular mass based on a mass : charge ratio. This single analysis cannot detect all modified species. As a simple example, TFPI dimers (which are aggregated, modified species) upon ionization in the electrospray analysis can have twice the charge as a TFPI monomer (an unmodified species), while also having twice the mass. The particular modification of dimerization (or higher level aggregation) therefore will not affect the mass to charge ratio of all dimers (or higher aggregates). At least some modified species will therefore be indistinguishable from (*i.e.*, considered "homogeneous" to) unmodified TFPI monomers in the electrospray analysis.

Applicants previously provided evidence of such shortcomings of the electrospray analysis, namely "Interpreting Electrospray Mass Spectra" at http://www.ionsource.com/tutorial/spectut/, page 2. As the Office Action acknowledges, this evidence shows that dimer formation during the analysis can interfere with the interpretation of results. Office Action at Page 5, lines 1-3. The Office Action, however, does not appreciate that

this interference results from the masking, by dimers formed during the procedure, of those already present in the sample. Electrospray therefore cannot robustly determine the percentage of dimers in samples having a tendency to aggregate.

In any event, the main point of the submitted evidence was to show why reliance on a mass : charge ratio of the electrospray analysis can result in a gross under-representation of modified species, and particularly aggregates such as dimers. This is apparent from the simple hypothetical example described on pages 2-3, where one compound having double the mass of another (*e.g.*, a dimer having a mass of 2000 and a monomer having a mass of 1000) can be ionized to the same mass : charge ratio and therefore display the same m/z peak.

Other analyses discussed in Diaz-Collier similarly cannot detect all modified species recited in the pending claims, namely oxidized, carbamylated, deamidated, cysteine adduct, aggregated, and/or misfolded TFPI molecules. As explained above, Diaz-Collier does not even address these modified species. Diaz-Collier instead deals with improving the activity of TFPI obtained from *E. coli* expression systems. Diaz-Collier favors *E. Coli* over mammalian cell hosts due to greater "homogeneity" of the TFPI product (*i.e.*, reduced phosphorylation, proteolytic internal cleavages, and glycosylation).

In contrast to Diaz-Collier, Gustafson, and other prior art TFPI preparation methods, Applicants have now discovered a particular sequence of operations after refolding (namely SP-Sepharose fast flow (FF) chromatography, a first concentration/diafiltration step, Q-Sepharose high performance (HP) chromatography, butyl hydrophobic interaction chromatography (HIC), SP-Sepharose high performance (HP) chromatography, and a second concentration/diafiltration step) that provides TFPI and TFPI analog preparations with the claimed properties, including less than about 12% modified species. There simply no basis to conclude that the TFPI preparations

disclosed in Diaz-Collier or other prior art references have less than about 12% of modified species, as recited in the pending claims.

Reconsideration and withdrawal of the rejections under 35 U.S.C. § 102 are respectfully requested.

The Rejection of Claim 19 under 35 U.S.C. § 103

Claim 19 is rejected as obvious over Diaz-Collier in view of Chen *et al.*, U.S. Patent No. 6,525,102 ("Chen"). Applicants respectfully traverse these rejections.

Claim 19 is directed to a pharmaceutical composition comprising a plurality of ala-TFPI molecules. Less than about 12% of the ala-TFPI molecules are modified species. Modified species have one or more of the following modifications: oxidation, carbamylation, deamidation, cysteine adducts, aggregation, and misfolding. The pharmaceutical formulation comprises 20 mM sodium citrate, 300 mM L-arginine, and 5 mM methionine, at pH 5.5.

A *prima facie* case of obviousness requires that the prior art reference (or references when combined) teach or suggest all the claim limitations. *In re Royka*, 490 F.2d 981, 985, 180 U.S.P.Q. 580, 583 (C.C.P.A. 1974) (emphasis added). For the same reasons given above with respect to the rejections under 35 U.S.C. § 102, Diaz-Collier neither describes (expressly or inherently) nor suggests a TFPI or ala-TFPI pharmaceutical composition "wherein less than about 12% of the ala-TFPI molecules are modified species [oxidized, carbamylated, deamidated, cysteine adduct, aggregated, and/or misfolded]" as recited in claim 19. Chen fails to cure this deficiency of Diaz-Collier, for the reasons stated in the Amendment and Response filed May 23, 2006.

In contrast to the disclosures of Diaz-Collier and Chen, Applicants have discovered a "purification method [that] produces preparations of TFPI or TFPI analog molecules that contain fewer modified TFPI or TFPI analog species than previous purification methods . . .The purification is of TFPI or TFPI analog is largely achieved after the folding step by a sequence of chromatography operations." Specification, paragraphs [63] and [64]. The sequence of chromatography operations, as set forth in detail throughout Applicants' specification, is not found in the prior art. The purification method disclosed by Applicants results in TFPI or TFPI analog compositions having the claimed, patentably distinct characteristics.

Reconsideration and withdrawal of the rejections under 35 U.S.C. § 103 are respectfully requested.

CONCLUSION

In view of the above remarks, all pending claims of this application are believed to be in condition for allowance. Acknowledgement of the same is respectfully requested. This response is believed to completely address all of the substantive issues raised in the Office Action dated August 8, 2006.

Please continue to direct all correspondence in this application to Chiron Corporation, Intellectual Property Dept., R440, 4560 Horton Street, Emeryville, CA 94608-2916.

Respectfully submitted,

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Date: November 8, 2006

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